SYNTHESIS AND SPECIFIC TRITIUM LABELLING OF THE BEHAVIOURALLY ACTIVE OCTAPEPTIDE DES-9-GLYCINAMIDE-LYSINE-VASOPRESSIN IN TWO DIFFERENT AMINOACID RESIDUES*

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Received October 20, 1977

SUMMARY

The specific labelling of the octapeptide,des-9-glycinamide--8-lysine-vasopressin in the tyrosine[2] and phenylalanine[3] residues with tritium is described. The 3.5-dibromotyrosine and 4-chloro--phenylalanine containing precursor peptides for labelling were synthesized by solid phase peptide synthesis. These peptides were catalytically tritiated giving the labelled derivatives with high specific radioactivity.

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The pituitary nonapeptide hormone,vasopressin has an important neurotropic action in mammals (1). Lande et al. (2) have found that the octapeptide des-9-glycinamide-[8-lysine]-vasopressin (DG-LVP) while having none of the endocrine effects of LVP, retains this central activity of the hormone. Based on observations of Lawler and duVigneaud (3), deWied et al. (4) have shown that tryptic

* The nomenclature used in the text is in accordance with the IUPAC-IUB Rules on Biochemical Nomenclature:Biochem.J.126:773 (1972) and J.Biol.Chem. 242: 555 (1967). Abbreviations used:Dbt: 3,5--dibromo-L-tyrosine,Cpa:4-chloro-L-phenylalanine, DMF:dimethyl-formamide, BOC:t-butyloxycarbonyl, DCC:dicyclohexylcarbodiimide, TFA:trifluoroacetic acid, TEA:triethylamin, Bzl:benzyl

0362-4803/78/0015-0163$01.00

digestion of LVP yields the same compound, DG-LVP. This octapeptide also stimulates conditioned avoidance acquisition in hypophysectomized rats (5), and delays extinction of active avoidance behaviour in intact rats. Synthetic DG-LVP has also been found to possess all these activities (6). The octapeptide also attenuates the puromycin-induced amnesia in tested animals (7,8). Since DG-LVP is free of the endocrine effects of LVP, it seems to be an ideal substrate for studying the neurotropic action of the hormone. Radiolabelled material would allow further studies on the binding and mechanism of action of this peptide. For this reason, we have prepared two differently labelled DG-LVP octapeptides.

The precursor peptides for tritium-labelling: 3,5-dibromotyrosine^2-DG-LVP (Dbt^2-DG-LVP) and 4-chlorophenylalanine^3-DG-LVP (Cpa^3-DG-LVP) were synthesized by solid phase peptide synthesis on conventional styrene-divinylbenzene copolymer resins. The peptides were cleaved from the resin and deprotected in liquid hydrogen fluoride, followed by oxidation of the disulfide bond. The peptides were purified by gel filtration.

Reduction of the precursor peptides by carrier-free tritium gas yielded specifically labelled octapeptides with relatively high specific radioactivity. Tyrosine-labelled preparations had specific radioactivities between 8 and 10 Ci/mmole, while phenylalanine-labelled batches were found to have specific activities between 3.5 and 5 Ci/mmole.

Experimental Section

All amino acids were of the L-configuration. BOC-derivatives and activated esters were prepared according to known procedures. The purities of amino acid derivatives as well as that of peptides were tested by thin layer chromatography. TLC's were ddveloped on Merck DC-Fertigplatten Kieselgel 60 . R_f values refer to the following solvent systems: R^1, n-butanol - acetic acid - water 8:5:4; R^2_f, n-butanol - acetic acid^f- water 4:1:1; R^3_f, n-butanol - pyridine- - acetic acid - water 15:10:3:6.

All reagents used for solid phase peptide synthesis were of analytical grade, while solvents were freshly distilled before use.

Column chromatographic elutions were followed by continuous monitoring of UV absorption of the eluent at 254 and 280 nm with LKB Uvicord 8300 and ISCO UA-5 photometers.Tritiations were carried out in a special vacuum manifold described elsewhere (9). Radioactivity determinations were carried out by liquid scintillation counting in Beckman LS-355 and Packard Tri-Carb 2425 equipment. Radiochromatograms were evaluated in a Nuclear Chicago Actigraph III scanner.

Amino acid analyses were determined im samples hydrolyzed by 6N HCl at 105° for 24 hrs (or 48 hrs for resin-peptides) on JEOL JLC-5 an Chinoin Lyz 75 amino acid analyzers. UV spectra were scanned in Beckman Model 25 spectrophotometers.

Boc-Lys (Z)- Resin

8 g chloromethylated BIO-BEADS SX-1 resin (capacity:1.1 mmol/g) was suspended in 50 ml dimethylformamide. 1.28 g (6 mmol) Boc-Lys(Z)-OH and 0.84 ml (6 mmol) triethylamine were added,dissolved in 15 ml DMF. The reaction mixture was stirred for two days at room temperature. Then the substituted resin was filtered off and thoroughly washed with DMF,ethanol, acetic acid and methylene chloride, finally dried. Yield: 10.55 g.

The degree of substitution was found to be 0.24 mmol/g by amino acid analyisis.

Z-Cys(Bzl)-Dbt-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys (Z)-Resin

1.5 g Boc-Lys(Z)-Resin was the starting material of the synthesis of the protected octapeptide-resin. Boc-amino acids were coupled in the presence of DCC, with the exception of Asn and Gln, which were coupled in the form of their p-nitrophenyl esters, while Dbt was coupled as its pentachlorophenyl ester. The synthesis plan followed the one described in the previous paper.

After the last coupling cycle the resin was thoroughly washed with dimethylformamide and methylene chloride, and dried in the dessicator. Yield:1.88 g.

H-Cys-Dbt-Phe-Gln-Asn-Cys-Pro-Lys-OH

1.8 g protected peptide-resin was suspended in 0.8 ml anisol. 20 ml anhydrous hydrogen fluoride was distilled onto the material and the reaction mixture was stirred for one hour at 0^{o}. HF was distilled off and the material was powdered with ether. This was dissolved in 500 ml 0.2 M acetic acid, its pH was adjusted to 6.8 with 0.5 M NH_4OH solution and aerated for 4 hrs. Afterwards the solution was stirred with BIO-RAD AG-3x4 ion exchange resin and lyophylized. The peptide was chromatographed by gel filtration on Sephadex G-15 column (2x90 cm) in 50 % acetic acid. The main component was further filtered through G-15 column (1.2x110 cm) in 0.2 M acetic acid.The fractions containing the peptide were pooled and lyophylized to give 138 mg homogeneous octapeptide.
R_f^1:0.60, R_f^2: 0.38. Amino acid analysis:Asp 1.01, Glu 1.03, Pro 0.94, Phe 1.00, Lys 1.06, Dbt 0.92.

[Tyr-3.5.-^{3}H]^{2} , des-9-glycinamide,8-lysine-vasopressin

5 mg Dbt^2-DGLVP was dissolved in 0.5 ml 1 % acetic acid. 50 mg Pd/Al_2O_3 catalyst was added and the peptide was tritiated at a pressure of 500 torr. The reaction mixture was stirred for 30 min at room temperature. Then the catalyst was removed by Millipore filtration and the solution was evaporated to dryness. 3x80 ml water was distilled from the material to remove exchangeable tritium. The residue was dissolved in 1.5 ml 0.2 M acetic acid and chromatographed on Sephadex G-15 column (1.1 x 18 cm) eluting with the same solvent. The fractions containing the octapeptide were pooled and lyophylized. A stock solution of 10 ml was prepared from the material. Peptide content: 220 µg/ml (by photometric assay). Radioactive concentration:1.75 mCi/ml (by LSC). Specific radioactivity: 8.04 Ci/mmole.

The material proved to be chemically and radiochemically homogeneous by TLC: R_f^2:0.29, R_f^3: 0.30. Amino acid analysis: Asp 1.02, Gln 1.01, Pro 0.93, Tyr 0.98, Phe 1,00, Lys 0.99.

H-Cys-Tyr-Cpa-Gln-Asn-Cys-Pro-Lys-OH

The synthesis followed the one described above except Boc-Cpa-OH and Boc-Tyr(Bzl)-OH were both coupled with DCC. 2.4 g peptideresin was deprotected and cleaved in liquid hydrogen fluoride followed by cyclization in the same fashion as described for the Dbt^2-analog. The lyophylized crude material was chromatographed on Sephadex G-15 columns (2x92 cm and 1.2x105 cm) in two steps yielding 144 mg homogeneous octapeptide.
R_f^1: 0,58, R_f^2: 0.34
Amino acid analysis:Asp 1.07, Glu 1.08, Pro 0,97, Tyr 0.96, Lys 1.02, Cpa 0.99.

[Phe-4-^{3}H]^3, des-9-glycinamide, 8-lysine-vasopressin

5 mg Cpa^3-DG-LVP octapeptide was dissolved in 0.5 ml 1 % acetic acid. 50 mg 10 % Pd/Al_2O_3 catalyst was added and the material tritiated for 30 min at room temperature. Then the catalyst was removed by Millipore filtration. 3x80 ml water was distilled from the material, which was further purified by gel filtration on Sephadex G-15 column (1.1 x 22 cm) eluting with 0.2 M acetic acid. The fractions containing the octapeptide were pooled and lyophylized. The material was dissolved in 10 ml water.
Peptide content: 236 µg/ml (photometrically)
Radioactive concentration: 1.03 mCi/ml
Specific radioactivity:4,4 Ci/mmole
R_f^1: 0.54, R_f^2:0.29. The material proved to be homogeneous both chemically and radiochemically.
Amino acid analysis:Asp 1.03, Gln 1.02, Pro 0.95, Tyr 0.96, Phe 1.00, Lys 1.01.

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